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Antibacterial Potential of $SrBi_2Ta_2O_9$ Synthesized via Molten Salt Method

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ABSTRACT

One of the interesting properties of Aurivillius compounds is photocatalysts; therefore, they have the potential to be used as antibacterials. However, the study of the antibacterial properties of Aurivillius compounds is still very limited, therefore it's important to conduct research on it's properties. Our research aims to study the photocatalytic activity of $SrBi_2Ta_2O_9$ compounds synthesized by molten salt method (using NaCl/KCl salt) as antibacterial. The diffractogram of the sample shows that the $SrBi_2Ta_2O_9$ was successfully synthesized, but an impurity phase was formed as Bi_2O_3, Ta_2O_5, and $SrBi_{2.83}Ta_5O_{15}$. The SEM images show that the morphological shape obtained is plate-like, which still has agglomeration with particle size distribution at range 20-80 μm. Plot tauc shows that the band gap energy of $SrBi_2Ta_2O_9$ compounds is 2.8 and 3.06 eV, respectively. The antibacterial activity test results showed that the $SrBi_2Ta_2O_9$ compound can inhibit staphylococcus bacterial growth under and without light radiation. It indicates that there are two possible mechanisms related to the inhibition of bacterial growth i.e: (a) photocatalysis, and (b) toxicity properties.

Key word: $SrBi_2Ta_2O_9$, molten salt synthesis, photocatalyst, antibacterial

INTRODUCTION

Pathogenic microorganisms have been a serious threat to human health. Pathogen microorganism such as bacteria, viruses, and protozoa have caused millions of deaths in the community each year due to diseases like diarrhea, trachoma, and cholera transmission, therefore the efforts are needed to solve it [1]. Many methods have been developed and used to handle pathogenic microorganisms. One of the most promising strategies to inhibit bacterial growth is photocatalysts [2]. In addition, the photocatalyst have many advantages such as low cost, efficient, and environmentally friendly [3]

One class of compounds reported to have excellent photocatalytic properties is the Aurivillius family of compound. The Aurivillius compound has the molecular formula $(Bi_2O_2)^{2+}$ $(A_{n-1}B_nO_{3n+1})^{2-}$ which is composed of a perovskite layer $(A_{n-1}B_nO_{3n+1})^{2-}$ and separated from the $(Bi_2O_2)^{2+}$ layer. Cation A is occupied by large ionic radii such as Sr^{2+}, Ca^{2+}, and Ba^{2+}, and cation B is occupied by smaller ionic radii such as Ti^{4+}, Nb^{5+}, W^{6+}, Ta^{2+} [4, 5]. Many researchers reported that some Aurivillius compounds have photocatalyst properties such as

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$Bi_4Ti_3O_{12}$, Bi_2WO_6, and $SrBi_4Ti_4O_{15}$ [6-8]. On other hand, the application of Aurivillius photocatalyst compound as antibacterial agents has been reported by several researchers [9]. Ren et al. (2009) reported that Bi_2WO_6 can inhibit *Escherichia coli* growth through photocatalysts [10].

$SrBi_2Ta_2O_9$ is a two-layer family of Aurivillius compounds with photocatalyst properties and having a band gap energy of 3.64 eV [11]. The photocatalytic activity of $SrBi_2Ta_2O_9$ compounds has also been reported by previous researchers. Rouf et al. (2021) reported that the $SrBi_2Ta_2O_9$ compound can degrade methylene blue dye under visible light [12]. Meanwhile, Li et al. (2022) reported the photocatalytic activity of $SrBi_2Ta_2O_9$ for the removal of NO [13]. However, the antibacterial capability of $SrBi_2Ta_2O_9$ compounds through photocatalytic mechanisms has yet to be reported.

Several factors influence the ability of photocatalyst activity, two of which are morphology and particle size [14, 15]. Many researchers reported that the Aurivillius compound with a plate-like/sheet particle morphology have excellent photocatalytic activity [16, 17]. One of the synthesis methods that can control morphology and particle size is molten salt method [18]. In addition, many researchers reported that molten salt method can produce plate-like/sheet Aurivillius compounds [19-21]. Zhao et al. (2014) reported successfully synthesizing $Bi_4Ti_3O_{12}$ [20] compounds by molten salt method (using Na_2SO_4/K_2SO_4) and obtained plate-like particles. Meanwhile, Wu et al. (2021) reported successfully synthesizing $CaBi_2Ta_2O_9$ compounds using the molten salt method and obtained nano plate-shaped particles with a particle size of 1.7 µm [22]. On other hand, Afqir et al. (2022) reported successfully synthesizing Gd doped-$SrBi_2Ta_2O_9$ compounds using the molten salt method ($NaNO_3$-KNO_3) and obtained plate-like particles [23]. The stages of forming Aurivillius compounds with a plate-like morphology include: (1) solid reaction and nucleation, (2) plate-like structure formation, (3) diffusion and edge nucleation, (4) diffusion and epitaxial growth [20].

One of the Aurivillius compounds that has been reported to have photocatalytic properties and used as an antibacterial is Bi_2WO_6 [24]. However, the investigation on the antibacterial properties of the Aurivillius compound is still limited especially study of the antibacterial properties of $SrBi_2Ta_2O_{12}$ compounds obtained from molten salt method. Therefore, in this research, we synthesized $SrBi_2Ta_2O_9$ compounds by molten salt and studied their photocatalyst properties to inhibit bacteria growth (as antibacterial compound). And in this studied, we used gram-positive bacterial *Staphylococcus aureus*.

EXPERIMENT

Materials

The materials used were $SrCO_3$ (Sigma Aldrich, 99% powder), Ta_2O_5 (Sigma Aldrich, 99% powder), Bi_2O_3 (Himedia, 99% powder), TiO_2 (Sigma Aldrich, 99% powder), NaCl (CDH), KCl (Merck, 99% powder), $AgNO_3$ (Merck), Acetone (Merck), distilled water, nutrient broth (Merck), nutrient agar (Merck).

Synthesis

In this research, 4 g amount of $SrBi_2Ta_2O_9$ compound was synthesized. NaCl and KCl salt were used with a mole ratio of 1:1. Meanwhile the mole ratio between product compound $SrBi_2Ta_2O_9$ and mixed salt NaCl/KCl is 1:7. The precursor and salt requirements were calculated stoichiometrically. Firstly, precursors and salts were mixed in mortar agate and ground for 3 hours. Acetone was added to make more homogenization while in the grinding process, and the homogenized mixture was obtained. The mixture was calcined at 750 and

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820°C for 6 hours. Then, the sample is removed from the furnace and washed using hot water to remove its salt content. The residual salt in the sample is identified with a silver nitrate ($AgNO_3$) solution [21]. If no white residue is found, that indicates the salt content is successfully removed from the sample product.

Characterization

The characterization techniques were used: (a) *X*-ray diffraction (XRD) aims to analyze the compound product phase and crystal structure. Measurements were carried out at room temperature using Cu Ka (40 kV, 20 mA) in the range 2θ= 10-80° (b) scanning electron microscope (SEM) is used for morphological analysis and particle size distribution calculations (c) ultraviolet-visible diffuse reflectance spectroscopy (UV-Vis DRS) is used to obtain reflectance spectra, which are then calculated using the Kubelka-Munk equation to determine the band gap energy aims to determine the band gap energy. The UV-Vis DRS measurement at range 200-800 nm

Antibacterial test

The stages of the antibacterial test include: (a) Preparation of Nutrient Agar (NA) Media (b) Preparation of Nutrient Broth (NB) Media (c) Bacterial Rejuvenation (d) Preparation of Inoculum (e) Antibacterial Activity of $SrBi_2Ta_2O_9$ Test (f) Viability of *S. aureus* Calculation. The detailed procedure is explained below.

Preparation of nutrient agar (NA) media

Media preparation was carried out by preparing 2 g of nutrient agar (NA) 100 mL of distilled water while heating to boiling, then transferring it to an Erlenmeyer and sterilizing it in an autoclave at 121°C for 15 minutes. The media was left at room temperature to solidify in an inclined position [25].

Preparation of nutrient broth (NB) media

Nutrient Broth (NB) was used for making bacterial inoculum. NB was made by dissolving 0.8 g of NB in 100 mL of distilled water, then putting it in Erlenmeyer and covering it with cotton. Then, the suspension was heated to boiling and then cooled to room temperature; the media was sterilized in an autoclave for 15 minutes at 121 °C [26].

Bacterial rejuvenation

Bacterial rejuvenation was carried out using the scratch method. A pure culture of *S. aureus* was several oses, then inoculated by scratching on NA media aseptically, and then incubated at 37 °C for 24 hours.

Preparation of inoculum

The inoculum was made by moving several oses of *S. aureus* into 25 mL of NB, then incubated at 37 ^{0}C for 18 hours. The turbidity of the *S. aureus* cell inoculum is equalized to optical density (OD) 0.1 at a wavelength of 600 nm.

Antibacterial activity of $SrBi_2Ta_2O_9$ Test

The compound of $SrBi_2Ta_2O_9$ which had been weighed according to the treatment, namely 2.5, 5, and 10 mg, were put into 100 mL of NB then 10 mL of *S. aureus* inoculum was added. The samples were exposed to four UV lamp (commercial LED UV Spotlight Bulb 80

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LEDs 220V E27) with stirring for 120 minutes in a homemade photocatalyst reactor. The test was also carried out using $SrBi_2Ta_2O_9$ without exposure UV lamp. As a control, the growth of *S. aureus* without treatment was also calculated and placed in an open space.

Viability of *Staphylococcus aureus* calculation

Viability of *S. aureus* was calculated using the pour plate method. 1 mL of photocatalyst and control media were taken and diluted in 9 mL of sterile 0.85% NaCl from 10^{-1} to 10^{-7}. As much as 0.1 ml of the dilution results were planted in a petri dish in duplo and then NA was added. Petri dishes were incubated at 37°C for 24 hours and then the number of colonies were counted. Cell viability was expressed in colony forming units (CFU) per milliliter.

RESULT AND DISCUSSION

The synthesized compound of $SrBi_2Ta_2O_9$ was identified by matching its diffraction pattern with the standard data for $SrBi_2Ta_2O_9$ (Joint Committee on Powder Diffraction Standards/JCPDS No. 00-049-0609). The diffraction pattern of the $SrBi_2Ta_2O_9$ compound is depicted in Figure 1, and it can be seen that there is a conformity between the diffraction pattern of the sample and the standard. It indicates that the $SrBi_2Ta_2O_9$ compound is successfully synthesized. However, several additional peaks that did not match the JCPDS standard No. 00-049-0609 were still found. It shows that the impurity phase was formed and corresponds to diffraction peaks at 2θ: (a) 2θ=52.6°, which was identified as Ta_2O_5, (b) 2θ= 58°, which was identified as Bi_2O_3 (c) 2θ =29.5, and 37.8° which were identified as $SrBi_{2.83}Ta_5O_{15}$. The existence of precursors (Bi_2O_3 and Ta_2O_5) as impurities indicates that the reaction is incomplete. It also showed that the synthesis conditions are not suitable for the synthesis [27].

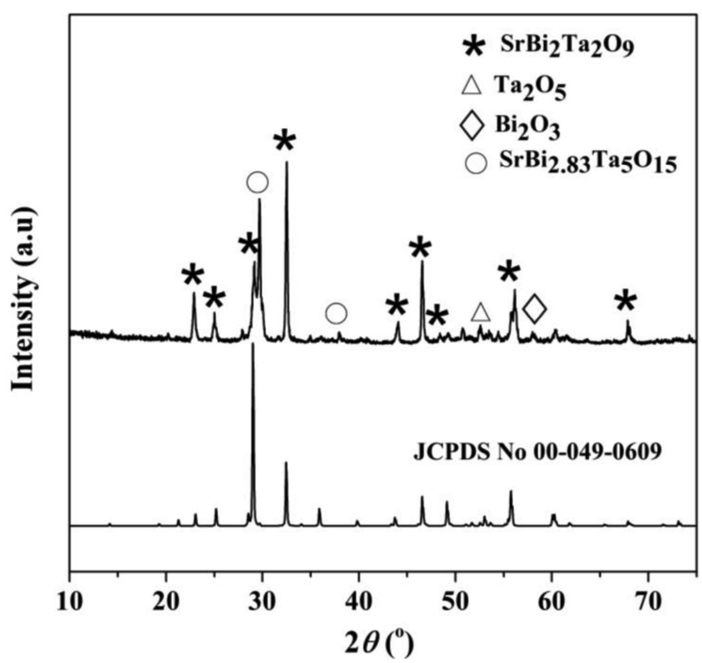

Figure 1. $SrBi_2Ta_2O_9$ diffractogram

Figure 2 showed the micrograph of the sample, and it can be seen that the morphology particle of the $SrBi_2Ta_2O_9$ compound is nonuniform plate-like and still found in many agglomerations. The plate-like particle morphology is a characteristic morphology of

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Aurivillius compounds that has been reported by previous researchers [20]. The particle morphology (Figure 2) synthesized through molten salt method is influenced by several factors, such as temperature and synthesis time, the type of salt, and the molar ratio of the targeted compound to the salt [28]. The synthesis temperature is crucial in forming morphology and agglomeration [18]. Therefore, the formation of agglomeration in this experiment is possible due to the relatively high synthesis temperatures used (750 and 820 ^oC). Figure 3 showed the particle size distribution, and the measurement results indicated that the obtained particle size within the range of 20-80 µm, and the most particles have a size between 40-50 µm.

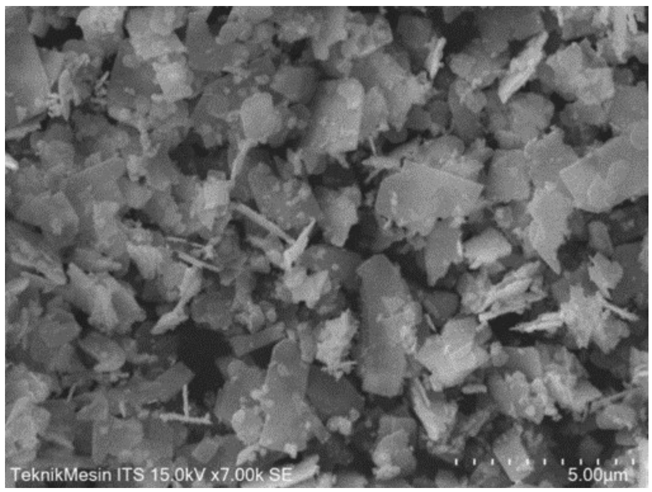

Figure 2. Micrograph of $SrBi_2Ta_2O_9$ compound

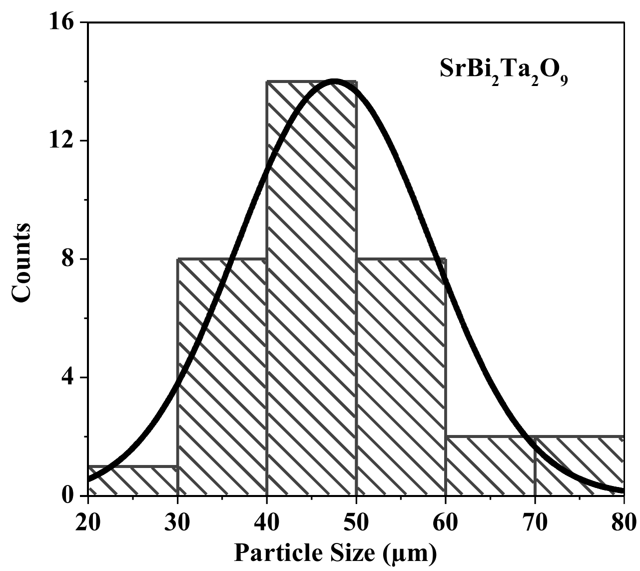

Figure 3. Particle size distribution of $SrBi_2Ta_2O_9$ compound

The DRS spectra of the $SrBi_2Ta_2O_9$ compound are shown in Figure 4 and then processed using the Kubelka-Munk equation (using the indirect-gap type) method to obtain band gap energy. The calculation results can be displayed as a Tauc plot (Figure 5), and it can be seen

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that the band gap energy of the sample is 2.8 and 3.06 eV. A previous study by Ma et al. (2023) reported that the band gap energy of $SrBi_2Ta_2O_9$ is 2.92 eV [29]. In addition, Jalili and Majidi (2008) suggested that the impurities affected the bandgap energy of MgO as result the existence of two band gap energies is probably due to impurities [30]. Therefore, it can be suggested that the bandgap energy of 2.8 eV belongs to $SrBi_2Ta_2O_9$. The band gap energy of $SrBi_2Ta_2O_9$ involved the electronic transition from *2p* O and *6s* Bi orbitals (valence band) to the *5d* Ta orbital (conduction band) [11]. The band gap energy value also showed that $SrBi_2Ta_2O_9$ can work in the ultraviolet and visible light spectrum.

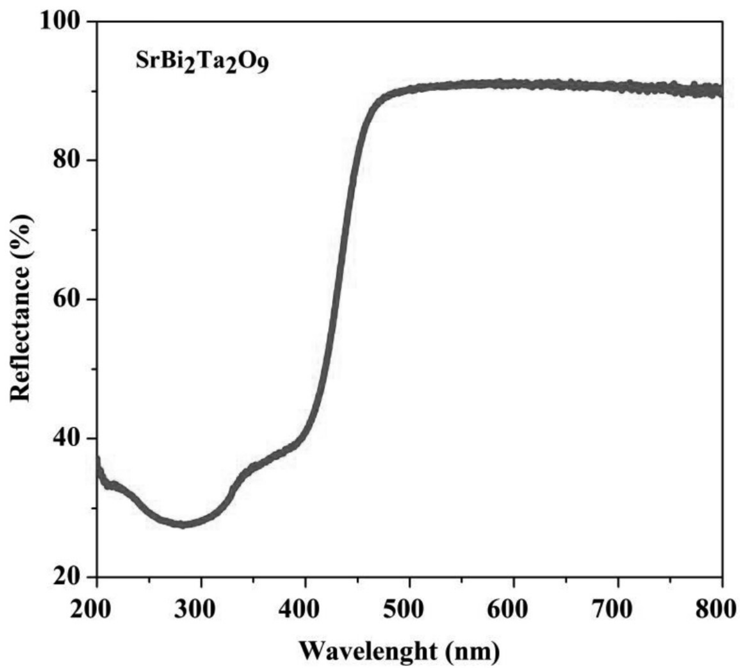

Figure 4. % Reflectance of $SrBi_2Ta_2O_9$ compound

The antibacterial activity of the $SrBi_2Ta_2O_9$ compound is depicted in Figure 6, and it can be seen that the bacterial growth reaches the highest number of 12.4 x 10^7 CFU/mL at open space conditions as control, which relates to the growth conditions are normally and did not disturb anything. The addition of the $SrBi_2Ta_2O_9$ compound with treatment exposed to light showed that bacterial growth was much smaller, namely at 2.5 mg and 5 mg of 7.3 x 10^7 CFU/mL and 4.3 x10^7 CFU/mL respectively. Meanwhile, the highest mass of $SrBi_2Ta_2O_9$ at 10 mg showed the viability similar to 5 mg namely 4.6 x 10^7 CFU/mL. This study showed that the viability of *S. aureus* was influenced by the mass of $SrBi_2Ta_2O_9$ used (Figure 6). The higher mass of $SrBi_2Ta_2O_9$ followed by the lower of the viability of *S. aureus* at 2.5 mg and 5 mg. The mechanism of inhibiting bacteria with photocatalysts through the process of irradiating $SrBi_2Ta_2O_9$ photocatalyst material with UV lamps produces $OH^{\cdot}$ radicals during the irradiation process that can damage cell walls and cause oxidative reactions in the cytoplasmic membrane, causing the release of fluids contained in the cell [31].

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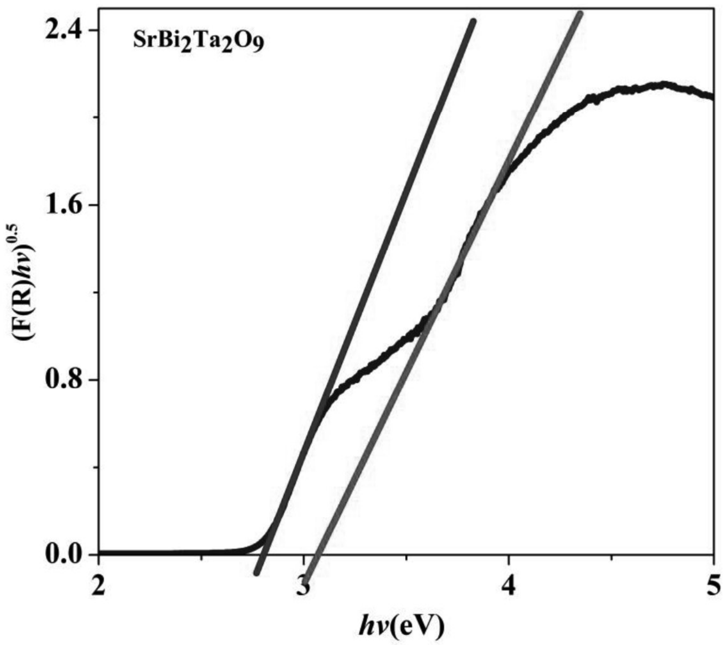

Figure 5. Tauc-plot of band gap energy of $SrBi_2Ta_2O_9$ compound

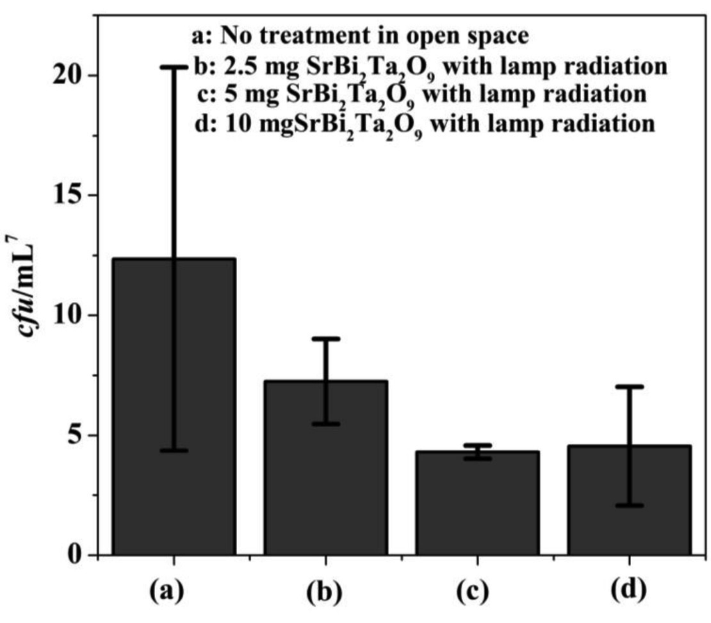

Figure 6. Antibacterial test of $SrBi_2Ta_2O_9$ compound with exposure UV lamp: (a) no treatment in open space (b) 2.5 mg $SrBi_2Ta_2O_9$, (c) 5 mg $SrBi_2Ta_2O_9$, and (d) 10 mg $SrBi_2Ta_2O_9$

Figure 7 showed bacterial growth decreases when the $SrBi_2Ta_2O_9$ compound is added without exposure to ultraviolet light radiation. It indicates that $SrBi_2Ta_2O_9$ compound can inhibit bacterial growth without a photocatalytic mechanism [32, 33]. The use of $SrBi_2Ta_2O_9$

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at 2.5 and 5 mg without radiation exposure showed the same inhibitory ability against *Staphylococcus aureus* with the viability of 7.0 CFU/mL, whereas at 5 mg it showed a higher inhibitory ability therefore the viability decreased to 5.0 CFU/mL. The inhibition (Figure 6 and 7) values also indicate that the antibacterial capability of the $SrBi_2Ta_2O_9$ compound is not strong. A comparison between Figure 6 and 7 showed that the inhibition capabilities do not differ significantly. It indicates that the antibacterial mechanism cannot explain in detail especially the photocatalyst contribution. However, many previous researchers also reported that metal oxides can be as antibacterial agent without photocatalyst mechanism. Dijaz et al. (2014) suggested that the the toxic properties of metal oxide compounds are caused by (a) The existence free metal ions as a result of the metal oxide dissolution process and (b) The rise of reactive oxygen species (ROS) on the surface of material [30]. Therefore, the study of antibacterial properties of $SrBi_2Ta_2O_9$ mechanism is urgent to reveal. In addition, high standard deviation value in antibacterial test indicates that the results obtained are highly fluctuating, so it would be beneficial to have a study on this topic using statistical analysis.

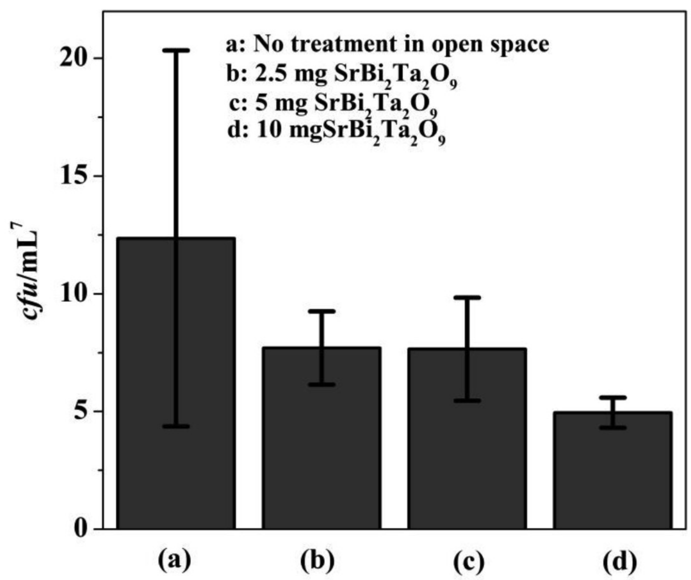

Figure 7. Antibacterial test of $SrBi_2Ta_2O_9$ compound without exposure UV lamp: (a) no treatment in open space (b) 2.5 mg $SrBi_2Ta_2O_9$ (c) 5 mg $SrBi_2Ta_2O_9$ and (d) 10 mg $SrBi_2Ta_2O_9$

CONCLUSION

The plate-like $SrBi_2Ta_2O_9$ compound was successfully synthesized using molten salt, but the impurities were still found i.e.: Ta_2O_5, Bi_2O_6, and $SrBi_{2.83}Ta_5O_{15}$. The sample obtained has two band gap energies of 2.8 and 3.06 eV due to the contribution from the presence of impurities. The results of the antibacterial activity test showed that the sample product can inhibit the growth rate of *S. aureus*. The antibacterial test results in both exposed and non-exposed UV light showed relatively similar. Therefore, the contribution of photocatalysis or the toxic properties of $SrBi_2Ta_2O_9$ cannot identified in detail.

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